REMARKS

Upon entry of this paper, claims 1-34 and 36-37 are canceled, and claims 35 and 38 are pending.

Applicants respectfully request entry of this paper to cancel claims withdrawn by the Examiner according to the Restriction Requirement dated June 26, 2006, and made final in the Office Action dated October 19, 2007. No new matter is entered. The withdrawn claims are canceled to simplify issues for appeal, and therefore, entry of the paper raises no new issues for consideration. Applicants reserve the right to pursue any canceled subject matter in one or more continuation, divisional, or continuation-in-part applications.

Interview Summary

Applicants acknowledge receipt of the Interview Summary mailed March 10, 2008, summarizing the telephone interview of February 26, 2008. Applicants' representatives Kenneth Sonnenfeld and Michael Willis thank Examiners Anoop Singh and Thaian Ton for the courtesy of the telephone interview. Claims 35 and 38 were discussed. Examiner Singh said that the specification contemplates various uses for the claimed products, and he is looking for a nexus between the uses described in the specification and the data presented in the examples. Applicants' representatives indicated that a written response to the Advisory Action would be filed.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 35 and 38 are rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement (Advisory Action, page 2, paragraph 3).

Applicants respectfully traverse on the basis that the claims are enabled through reduction to practice and in view of the state of the art. The claims are directed to a transgenic mouse and a cell obtained therefrom, as claimed in claims 35 and 38. The specification describes the use of knockout non-human animals and cells thereof for screening agonists or antagonists (see page 6, right column, lines 13-17 in the published application) and for elucidating mechanisms of response to CpG ODN (see page 6, left column, lines 15-17 in the published application), and such techniques are further exemplified in Experiment 4, resulting in data such as presented in Figures 5, 6, 7, and 8. Applicants believe nothing further is necessary for complying with the enablement requirement. However, in order to advance prosecution, Applications present additional arguments and evidence of enablement, as follows.

Assays With Knockout Mice And Their Cells Are An Enabled Use

The specification states that knockout mice and cells according to the invention "enables us to screen agonists or antagonists of the receptor proteins… or suppressing or promoting substances" (Specification as published, page 6, paragraph [0050], right column, lines 13-18). In other words, the specification describes assaying for immunomodulatory compounds acting through a particular signal transduction pathway. This is a use which, if enabled, satisfies the requirements of 35 U.S.C. § 112, 1st paragraph, particularly in view of the state of the art, and the fact that such an assay is reduced to practice in Experiment 4 in the specification. Applicants note that the claims are directed to knockout mice and cells thereof rather than methods of treatment.

Reduction To Practice Of How To Use The Claimed Invention In An Assay

Experiment 4 in the specification as filed compares the response in wild-type and knockout mice according to the invention to bacterial DNA having an unmethylated CpG sequence. Specifically, Experiment 4 provides data from a number of measurements comparing these responses.

First, the amount of inflammatory cytokines produced in peritoneal macrophages was measured. Macrophages from wild-type and knockout mice were prepared and co-cultured with CpG ODN. The concentrations of three cytokines produced in response to the CpG ODN were measured: TNF-α, IL-6, and IL-12 p40. The results are shown in Figure 5 in the specification. The results show that the macrophages from wild-type mice produce the three cytokines in response to CpG ODN, and the levels of the three cytokines can be increased by further stimulation with IFN γ. In contrast, the knockout mice produced no detectable levels of the three cytokines in response to CpG ODN alone or in response to CpG ODN in combination with IFN γ. The specificity of the response to CpG ODN was confirmed through the use of positive controls LPS or PGN, which caused production of similar levels of cytokine in both wild-type and knockout mice, providing evidence that the differential production of cytokines in response to challenge with CpG ODN was specific for CpG ODN. In other words, through the use of the knockout mice, Applicants have shown in Experiment 4 that the immune system has a specific response to CpG ODN (*i.e.*, production of cytokines by macrophages) that proceeds through a different pathway than the immune system response to LPS or PGN. In addition, Applicants have shown that LPS and PGN are not agonists of the same signal transduction pathway as CpG ODN.

Second, the proliferation of cells was measured in response to administration of

CpG ODN. Specifically, the response of spleen cells in wild-type and knockout mice was compared for both CpG ODN and LPS. Spleen cells of each type were stimulated with various concentrations of CpG ODN or LPS, and the results are shown in Figure 6 of the specification. The wild-type cells proliferate in response to either CpG ODN or LPS, but the knockout mice only proliferate in response to LPS. The specificity of the response is shown through the use of the positive control LPS. In other words, through the use of the knockout mice, Applicants have shown in Experiment 4 that the immune system has a specific response to CpG ODN (*i.e.*, proliferation of immune cells) that proceeds through a different pathway than the immune system response to LPS. In addition, Applicants have shown that LPS is not an agonist of the same signal transduction pathway as CpG ODN.

Finally, the amount of inflammatory cytokines produced in dendritic cells was measured. Immature dendritic cells from wild-type and knockout mice were prepared from bone marrow cells and co-cultured with either CpG ODN or LPS. The concentrations of the cytokine IL-12 p40 produced in response to either CpG ODN or LPS was measured. The results are shown in Figure 7. The wild-type dendritic cells produce cytokine in response to either CpG ODN or LPS, but the knockout mice only proliferate in response to LPS. Differential expression of various receptors on the surface of the dendritic cells is also shown in Figure 8. The specificity of the response is shown through the use of the positive control LPS. In other words, through the use of the knockout mice, Applicants have shown in Experiment 4 that the immune system has a specific response to CpG ODN (*i.e.*, production of cytokine by dendritic cells) that proceeds through a different pathway than the immune system response to LPS. In addition, Applicants have shown that LPS is not an agonist of the same signal transduction pathway as CpG ODN.

The Advisory Action states that

> An artisan would not know whether macrophage having reduced reactivity to unmethylated CpG sequence is due to TLR9 knockout or it is because of other compensatory factors.

(Advisory Action, page 2, lines 41-42). Applicants respectfully disagree with this statement as being incorrect. The use of LPS and PGN as positive controls (and the resulting similar responses of wild-type and knockout mice to LPS and PGN) provides evidence that the differential response to CpG ODN by wild-type and knockout mice is specific for a distinct signaling transduction pathway. Of course, there may be additional downstream elements in the pathway- Experiment 4 in the specification identifies the protein MyD88 as a mediating protein in the signal transduction pathway for response to CpG ODN. However, the use of the TLR9 knockout mice according to the invention identifies TLR9 as at least one required component in the response to CpG ODN by the immune system. One of ordinary skill in the art would recognize that each individual component in a signaling transduction pathway is important because it can serve as an additional entry point for modulation of the immune system, and the assay reduced to practice in Experiment 4 provides a screen for agonists and antagonists for modulating that component in the signal transduction pathway.

State Of The Art In How To Use Knockout Mice And Their Cells In Assays

The Advisory Action states

> It is apparent that instant specification has not provided any guidance as to how an artisan would have used the TLR knockout mouse in bacterial infection as described in this application. In absence of any specific teaching an artisan would have to perform undue experimentation to make and use of the invention. It is emphasized that there is no teaching as to how to use the claimed animals for any other purpose other than against bacterial infection.

(Advisory Action, page 2, lines 45-48). Applicants respectfully disagree with the Advisory Action for ignoring the use identified in the specification in paragraph [0050] as published- *i.e.*, for assays for agonists or antagonists in the immune system response to CpG DNA and the reduction to practice thereof in Experiment 4, and for failing to consider the state of the art at the time of filing.

Applicants note that the immunomodulation achieved in the assay in Experiment 4 (*i.e.*, stimulation of cytokine production by macrophages, proliferation of immune cells, and stimulation of cytokine production by dendritic cells) is functionally important, as was already known in the prior art at the time of the invention. For example, macrophages are cells within the tissues that originate from specific white blood cells. They are phagocytes, acting in both non-specific defense as well as specific defense of vertebrate animals. Their role is to phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or mobile cells, and through release of cytokines, to stimulate other immune cells to respond to the pathogen. In addition, spleen cells are regarded as the center of activity of the reticuloendothelial system, which is part of the immune system. Furthermore, the prior art teaches that dendritic cells are essential for the initiation of primary immune responses in T cells *in vivo* (see Jakob,[1] page 3042, right column, lines 18-20). Finally, Krieg[2] demonstrates that activation of the immune response with CpG DNA heightens the resistance to bacterial *L. monocytogenes* infection (see page 2429, left column, lines 11-13). In other words, it was recognized in the art that immunomodulation (*i.e.*, stimulation of cytokine production by macrophages, proliferation

[1] Jakob et al., "Activation of Cutaneous Dendritic Cells by CpG-Containing Oligodeoxynucleotides: A Role for Dendritic Cells in the Augmentation of Th1 Responses by Immunostimulatory DNA," Journal of Immunology, vol. 161, pages 3042-3049, 1998.

[2] Krieg et al., "CpG DNA Induces Sustained IL-12 Expression In Vivo and Resistance to *Listeria monocytogenes* Challenge," Journal of Immunology, vol. 161, pages 2428-2434, 1998.

of immune cells, and stimulation of cytokine production by dendritic cells) is important in the immune system response to bacterial infection.

As an example of the state of the prior art with respect to assays, Krieg teaches methods of assaying with knockout mice. In particular, Krieg uses knockout mice to teach that resistance to bacterial infection with *Listeria monocytogenes* can be achieved by immune activation with CpG DNA (see page 2432, right column, lines 18-20). For his studies, Krieg uses IFN-γ knockout mice purchased from The Jackson Laboratory (see page 2429, left column, lines 20-22). With the IFN-γ knockout mice, Krieg demonstrates that CpG DNA activates the immune response and heightens the resistance to bacterial *L. monocytogenes* infection (see page 2429, left column, lines 11-13). In explaining his results, Krieg relies on a number of experiments using the IFN-γ knockout mice (see page 2433, right column, lines 1-35). **However, the IFN-γ knockout mice are not sufficient to assay for TLR9 agonists or antagonists as described in the instant specification**: Krieg specifically states that "[f]urther studies will be required to identify the tissue and cellular source of the sustained IL-12 response to CpG DNA," (page 2433, right column, lines 15-17). In performing such further assays for agonists or antagonists of the sustained IL-12 response with the knockout mice according to the invention, one of ordinary skill in the art would not require undue experimentation in order to use the invention because the state of the art and the instant specification demonstrate how to use knockout mice in performing such assays, *i.e.* by following the methodology of Experiment 4 with mice and their cells according to the invention rather than the IFN-γ knockout mice of the prior art.

<u>Nexus Between Immunomodulation By CpG ODN And Treatment Of Infection</u>

The immunomodulation achieved in the assays of Experiment 4 is not just theoretical. The prior art recognized that such immunomodulation has real-world importance for the treatment of infection. As mentioned above, Krieg demonstrated that activation of the immune response with CpG DNA heightens the resistance to bacterial *L. monocytogenes* infection (see page 2429, left column, lines 11-13). In addition, as mentioned in the previous response, it was already reported by Zimmerman[3] that immunomodulation with CpG ODN could treat and cure *Leishmaniasis major* infection.

In response to Zimmerman, the Advisory Action states that the teaching of Zimmerman "provides evidence of role of TLR9 against parasitic infection but fails to establish any nexus with bacterial infection," (Advisory Action, page 2, lines 25-26). The Advisory Action also states that the specification "discloses no nexus between TLR9 and any known bacterial pathological state," (Advisory Action, page 2, lines 32-33). However, such teachings in the specification are not necessary because they merely restate what was already known in the prior art at the time of filing, *i.e.*, the prior art recognized the nexus between Zimmerman and bacterial infection.

In one example of the prior art recognizing the nexus between Zimmerman and bacteria, Elkins[4] teaches that the results of Zimmerman with intracellular pathogens are **confirmed and extended** to bacterial infection with *Francisella tularensis* live vaccine strain (LVS) and *Listeria monocytogenes*. The two bacteria studied by Elkins are bacteria that are studied in the art as models for bacterial infection (Elkins, page 2291, right column, lines 6-11).

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[3] Zimmerman et al., "Cutting Edge: CpG Oligodeoxynucleotides Trigger Protective and Curative Th1 Responses in Lethal Murine Leishmaniasis," Journal of Immunology, vol. 160, 3627-3630, 1998, considered by the Examiner as indicated by the initialed IDS returned with the Advisory Action

[4] Elkins et al., "Bacterial DNA Containing CpG Motifs Stimulates Lymphocyte-Dependent Protection of Mice Against Lethal Infection with Intracellular Bacteria," Journal of Immunology, vol. 162, pages 2291-2298, 1999.

Specifically, Elkins presents data showing protection against bacterial infection (*F. tularensis* LVS and *L. monocytogenes*) with CpG-ODN, and in the Discussion section, teaches that the results of the studies with bacteria "**confirm and extend** other recent observations concerning the ability of bacterial DNA to modulate infection by intracellular pathogens," (Elkins, page 2296, right column, lines 5-7, emphasis added). Elkins specifically discusses the results of Zimmerman to show that Zimmerman is **confirmed and extended** to bacterial infection (Elkins, page 2296, right column, line 7 through page 2297, line 4). Therefore, the nexus between Zimmerman and bacteria was known in the art prior to the filing date of the instant application, as shown by Elkins.

Additional evidence that the nexus between Zimmerman and bacteria was known in the art can be seen in Wagner[5], which further verifies the nexus between Zimmerman and bacteria. Wagner teaches that CpG-ODN confer resistance against infection with *Listeria monocytogenes* (bacteria) and *Francisella tularensis* (bacteria) (see Wagner, page 149, lines 14-31). In addition, Wagner specifically teaches that the results of Zimmerman for *Leishmania major* "parallel, at least in part, those obtained in protection assays to *L. monocytogenes* and *F. tularensis*," (see Wagner, page 150, lines 1-3). Therefore, the nexus between Zimmerman and bacteria was known in the art prior to the filing date of the instant application, as shown by Wagner.

Summary

In summary, the prior art recognized immunomodulation with CpG ODN and its relevance for bacterial infection (for example, see Krieg, Elkins, and Wagner). The specification

[5] Wagner et al., "Immunostimulatory DNA sequences help to eradicate intracellular pathogens," Springer Semin Immunopathol, vol. 22, pages 147-52, June 2000.

as filed, including Experiment 4, provides the knockout mice to assay for agonists or antagonists acting through the same signal transduction pathway as CpG ODN, and teaches one of ordinary skill in the art how to use the claimed mice and their cells in such assays.

CONCLUSION

Based on the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims and allowance of this application.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **50-3732**, Order No. 14119.105010. In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **50-3732**, Order No. 14119.105010.

Respectfully submitted,
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